HEPATITIS AND LEUKEMIA: THEIR RELATION TO AUSTRALIA ANTIGEN

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In 1961 we discovered that patients who have received transfusions may develop precipitating antibodies against serum beta-lipoprotein.[1-3] The antisera found in these patients were used to define a system of inherited antigenic specificities on the serum low-density beta-lipoproteins.[4, 5] This was termed the Ag system, and a variety of specificities and alleles have been described.[6, 8] These antibodies were common in patients who received very large numbers of transfusions (for example about one third of thalassemia patients), and the antigens which reacted with these specificities were common in the general population. A search for additional precipitating antibodies was continued on the hypothesis that other antigenic systems might be discovered. In 1964 an antibody was detected in the serum of a transfused hemophilia patient which was clearly different from the lipoprotein precipitins previously found.[9] It reacted with only one serum in a panel of 24 against which it was tested; since the reacting serum was that of an Australian aborigine, the antigen was given the geographic name "Australia antigen."[10] Subsequent studies showed the antigen to be extremely rare in American populations but it was relatively common in several interesting diseases (Table I). It was found in acute myelogenous leukemia, chronic lymphocytic leukemia, and acute lymphocytic leukemia, but not in chronic myelogenous leukemia.[10-12] The antigen was also found in institutionalized patients with Down's syndrome (mongolism), who are known to have an increased risk of developing acute leukemia, in lepromatous leprosy, in thalassemia, and in other patients who had received large numbers of transfusions.[11-15] These frequencies were particularly impressive because of the failure to find the trait in many other disease groups

This study was supported by Public Health Service Research Grants CA-08069, CA-06551, CA-06927, and FR-05539 from the National Cancer Institute, Bethesda, Md., a grant from the World Health Organization, and an appropriation from the Commonwealth of Pennsylvania.

TABLE I. AUSTRALIA ANTIGEN DISTRIBUTION IN PATIENTS AND CONTROLS

	No. tested	*Au positive* No.	%
Controls (nonhospitalized "normal")	2,412	2	0.1
Hospital patients, serial admissions	1,055	2	0.2
Liver disease			
Hepatitis, acute viral	125	25	20
Infectious mononucleosis	67	0	0
Laennec's cirrhosis	52	0	0
Other liver disease	15	0	0
Leukemia			
Acute myelogenous	51	7	14
Chronic myelogenous	63	0	0
Acute lymphocytic	89	11	12
Chronic lymphocytic	49	5	10
Hodgkin's disease	16	2	13
Down's syndrome (institutionalized)	257	75	29
Transfused anemia patients	144	5	3
Solid tumors	95	0	0
Various other diseases	1,414	0	0

Addition detail is given in Blumberg *et al.*,[10] Sutnick *et al.*,[25] London *et al.*,[18] and Blumberg *et al.*[11]

tested and also because of its extreme rarity in apparently normal populations in the United States (Table I).

The antigen is detected by immunodiffusion techniques that use a micro-Ouchterlony pattern. The antiserum is placed in the center well of the pattern, and the sera which are to be tested for the presence of Australia antigen in the peripheral wells. A clear precipitin band forms between the center well and the peripheral wells containing sera with the antigen (Figure 1). Observations are made on the formation of the precipitin, and the slides are then washed,dried, and stained with azocarmine for the identification of faint reactions. Several hundred sera may be tested in a day by means of these techniques.[16] An important advance in our investigations was made when Liisa Melartin was able to produce antibody against Australia antigen in rabbits.[17] The animals were given injections of human sera that contained Australia antigen; antibodies formed within weeks or months without the use of adjuvants. The antisera were absorbed with the serum of an individual who did not have Australia antigen; this resulted in an antibody which re-

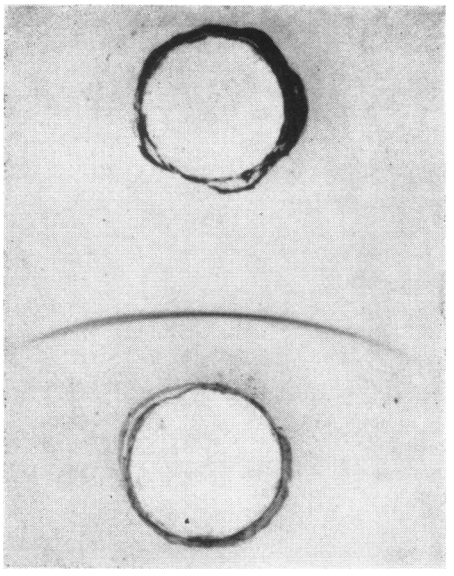

Fig. 1. Typical appearance of Australia antigen precipitin in double diffusion experiment. The antiserum (serum of a hemophilia patient) is in the upper well and the antigen (serum of leukemia patient) in the lower well. The precipitin band has been stained with azo-carmine.

acted only with Australia antigen. In most of our experiments both the human and rabbit antisera have been used. These techniques were further developed by C. Levene, who discovered additional specificities of Australia antigen which will be described later.

Australia Antigen Associated with Hepatitis

We proceeded with the analysis of the Australia antigen system along the lines developed for the lipoprotein polymorphism study. However, very early in the investigations the possibility of the infectious nature of this material became apparent,[10] and a clear association of Australia antigen with hepatitis was established.[11] In the course of surveying sera from presumed normal people we found two who had the antigen. We subsequently found that one of these individuals had hepatitis and the other had recently been transfused. We then tested the sera of 125 patients who either had or were suspected of having acute viral hepatitis; of these 20 per cent were positive for Australia antigen (Table I).[18]

Multiple samples taken during the course of acute viral hepatitis were available on 56 patients, including 15 patients in whom Australia antigen was detected in the first instance. Of the 15 patients, only two had the antigen when blood was tested several weeks or months later. The antigen had disappeared from the blood of others. This is in striking contrast to the persistence of the antigen in other conditions and populations; this will be discussed in greater detail later. From this it appeared that the antigen was detected, generally speaking, early in the illness but disappeared after days and weeks. Since only a single sample was obtained from the majority of the patients we tested there was a high probability of missing some positives. Hence, the 20 per cent positive frequency represents a minimum value. The antigen was not found in patients with infectious mononucleosis (who have a form of hepatitis),[19] hepatic cirrhosis, hepatoma, and a heterogeneous group of liver diseases (Table I). From this we concluded that the antigen was specifically found in patients with viral hepatitis and was not associated with liver disease of other etiology. There was a higher frequency of the antigen in patients with the diagnosis of posttransfusion hepatitis (34 per cent of 41 patients) compared to those classified as infectious hepatitis (13 per cent of 84 patients). There was no sex difference. The age distribution was striking in that the antigen was extremely rare in individuals under 20 but did not seem to vary in frequency in ages greater than 20. This again is in striking contrast to the age distribution found in other diseases and populations; it will be discussed later.

It now became important to determine if Australia antigen itself was an infectious agent associated with hepatitis. Several observations, which should be classified as "anecdotal," raised our suspicion that this might be so. A young lady who worked in our laboratory developed loss of appetite and dark urine, and was found to have a markedly elevated SGPT. She also had Australia antigen in her serum, but for only a single day. Although this could have been coincidence (it occurred during an "epidemic" year for hepatitis), it did raise the suspicion that she had contracted the disease as a result of her exposure to sera containing Australia antigen, i.e., that the antigen itself was infectious. Her hepatitis was mild and she recovered without incident after several weeks of bed rest.

An observation even more suggestive of infection and reproducibility of the "antigen" was the development of antibody against Aus-

tralia antigen in high titer in one of our colleagues who had been immunizing rabbits for more than a year with human sera containing Australia antigen. This investigator had never received a blood transfusion nor, to our knowledge, had he contracted clinical hepatitis; moreover, Australia antigen was never detected in any of his blood specimens. He did not knowingly inject himself with the sera containing the antigen although he came in frequent contact with it.

The third observation was on an individual who resided on Rongelap Atoll in Micronesia; from him we had obtained serum yearly for five years. In 1964 he had no detectable precipitin. In 1965 he received a single blood transfusion for the treatment of a bleeding ulcer after which he had antibody against Australia antigen. The next year the antibody had disappeared and Australia antigen was present. It reacted not only with our standard anti-Australia antiserum, but also specifically with the precipitating antibody found in his serum the preceding year. In this instance we presumed he had received the antigen in the transfused blood (Australia antigen would be expected to occur in about 7 per cent of Micronesian blood donors) and initially had developed an antibody against it. The finding of the antigen in the subsequent year suggests that there was an excess of infectious material relative to the antibody.

Viruslike Appearance of Australia Antigen Particles

All of these observations were consistent with the hypothesis that the antigen was an infectious, reproducing material but, of course, by their nature did not prove this. H. J. Alter had developed methods for the isolation of Australia antigen, but sufficient amounts for extensive studies were not available during the early years.[20] Subsequently, in cooperation with A. M. Prince of New York City we subjected sera that contained the antigen to ultracentrifugation on a sucrose gradient.[21] We used 10 to 30 per cent sucrose, and layered either 0.2 ml. or 0.6 ml. of serum containing the antigen on each gradient. The presence of the antigen in the gradient fractions was detected by the micro-Ouchterlony technique. The antigen was concentrated in a relatively small number of fractions (Figure 2), and these were, for the most part, free of contaminating serum proteins. The fractions with the highest concentration of Australia antigen were examined by M. E. Bayer by means of the electron microscope. They contained particles of about 20 μ

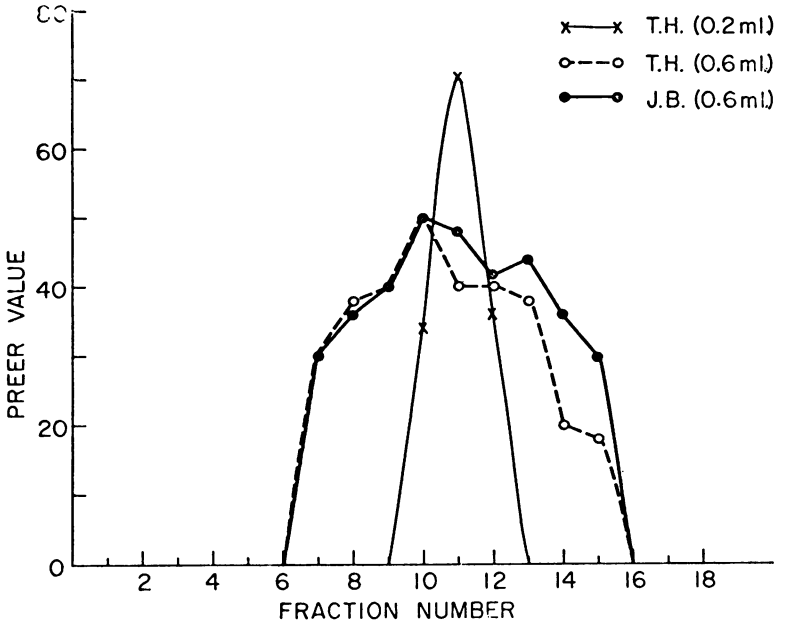

Fig. 2. Separation of Australia antigen by sucrose gradient density ultracentrifugation. The amount of Australia antigen is expressed in arbitrary Preer value units. The fraction numbers in which the antigen was detected are shown on the abscissa. The results of three experiments are shown: patient T.H., 0.2 ml. and 0.6 ml.; and patient J.B., 0.6 ml. Adapted from Bayer *et al.*[21]

diameter[21] (Figure 3). The surface appeared to be composed of knob-like "subunits" of about 70 A diameter. In some of the particles a central "core" was observed whereas in other particles the center appeared to be entirely filled with stain. Addition of specific anti-Australia antigen rabbit gamma-globulin resulted in agglutination of particles (Figure 3). An approximately quantitative relation was seen; addition of large amounts of gamma-globulin led to the formation of large aggregates while small concentrations yielded small aggregates. Rows of particles that formed short "strings" could sometimes be seen as well as what appeared to be two dimensional aggregates. The appearance of these particles is similar to that of the picorna virus group. Furthermore, they are about the size postulated for hepatitis virus.[22] However, the demonstration of particles is not sufficient evidence to identify Australia antigen as a virus and, obviously, additional experiments to determine the nature of these particles are required.

During the past 20 years many isolations of hepatitis virus have been

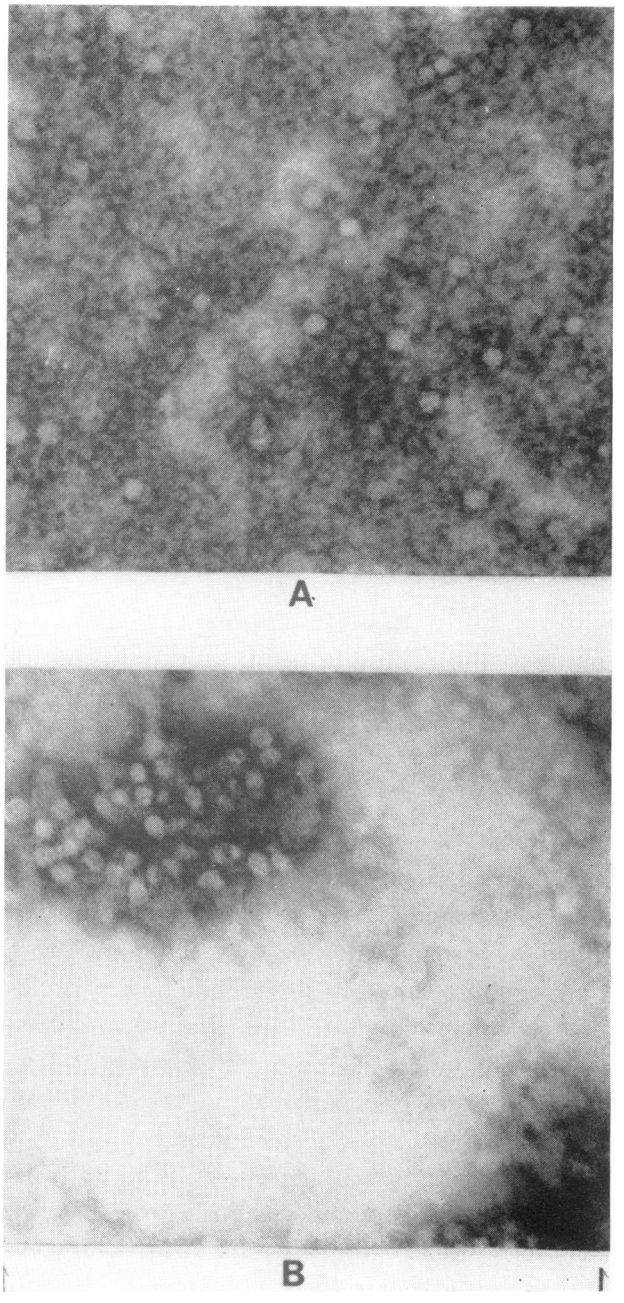

Fig. 3. *A*. Particles from the serum fraction containing Australia antigen, negatively stained. ×200,000. *B*. Australia antigen and specific antibody; note aggregation of particles. ×170,000. Adapted from Bayer *et al.*[21]

TABLE II. SGPT IN PERSONS WITH AND WITHOUT AUSTRALIA ANTIGEN*

		Unclassified mentally retarded	*Down's syndrome*	
		Au(0)	*Au(0)*	*Au(1)*
Institution 1	No.	17	63	38
	Median	16.3	14.2	38.2 ($p < 0.001$)
Institution 2	No.	112	79	34
(Institutions 1 and 2)	Median	14.6	15.4	39.4 ($p < 0.001$)
Total	No.	129	142	72
	Median	14.7	15.3	37.8 ($p < 0.001$)
Outpatients	No.		14	
	Median		11.5	
			Normal Au(0)	
Staff personnel (institution 2)	No.		114	
	Median		13.0	

*Adapted from Sutnick *et al.*[25]

reported. Apparently, none of these has been consistently reproducible; as a consequence "candidate" viruses for hepatitis are viewed, with good reason, with great suspicion. Consequently we have collected additional evidence bearing on the association of the antigen with hepatitis and we are searching for additional virologic information. This is reviewed in the following sections.

ASSOCIATION OF AUSTRALIA ANTIGEN WITH SGPT ELEVATION IN DOWN'S SYNDROME

Elevation of serum glutamic pyruvic transaminase (SGPT) is a sensitive indicator of damage to the liver cell, and it is usually elevated in clinical hepatitis.[23] We have found that Australia antigen is related to SGPT levels in institutionalized patients with Down's syndrome. Studies were carried out independently in two large institutions with nearly identical results.[24, 25] The combined study included 471 patients and controls. Down's syndrome patients with the antigen had mean and median levels of SGPT significantly higher than similar patients without the antigen (Table II). The median level in 72 patients with Australia antigen was 38, and in 142 patients without the antigen it was 15 units. None of these patients was jaundiced. SGPT levels were not elevated in outpatients with Down's syndrome, in other mentally retarded

patients in the same institutions, nor in the employees of one of the institutions; none of these had Australia anitgen. Liver biopsies were done on eight patients with Australia antigen with or without elevated SGPT. There were inflammatory changes compatible with the diagnosis of chronic anicteric hepatitis in all cases.

Association of Australia Antigen with Elevated SGPT in Cebu, the Philippines

By means of a case-control technique we compared individuals with and without Australia antigen in respect of SGPT levels in patients and in control subjects from Cebu. (The frequency of Australia antigen is high in the Philippines; this also will be discussed later.) In the initial study a total of 47 pairs were compared; i.e., the case was an individual with Australia antigen, the control was an individual of the same age, sex, diagnosis, and habitat without the antigen. The sera from both case and control were collected on the same day. In 29 pairs the SGPT was higher in the individuals with Australia antigen than in the controls, and in only 12 pairs was the SGPT higher in the controls. The likelihood that this is due to chance is less than 0.05. Taken with the other studies, this supports the hypothesis that the antigen is associated, at least in part, with both acute and chronic liver damage which is probably hepatitis.

Blood Donor and Transfusion Studies by Okochi

K. Okochi of the Blood Transfusion Service of Tokyo University Hospital has made extremely important observations on the relation of Australia antigen to hepatitis, and he has graciously allowed us to see his studies prior to their publication.[26, 27]

He confirmed that Australia antigen is associated with viral hepatitis;[11, 18] also, that the frequency of Australia antigen is higher in Japan (1 per cent) than it is in the United States (0.1 per cent).[10, 28] Further, he showed that patients transfused with blood containing Australia antigen could: 1) develop Australia antigen and hepatitis, or 2) develop antibody to Australia antigen, with no evidence of hepatitis. We interpret these data, combined with the earlier observations, to mean that Australia antigen is (or is closely associated with) an infectious material which can serve to transmit hepatitis.

Studies on Transfused Patients

We are currently studying a group of patients treated with frequent transfusions at the Jefferson Medical College Hospital for a variety of conditions: i.e., hemophilia, kidney failure, leukemia. Of 50 patients thus far tested 15 per cent were found to have Australia antigen. This includes nine patients undergoing chronic hemodialysis, of whom eight had Australia antigen. The median SGPT levels in those with Australia antigen is 81; in transfused patients without the antigen it is 19. This finding is consistent with the other studies showing an association of elevated SGPT with Australia antigen. It further demonstrates the importance of transfusion as a method of transmitting the agent.

Further Studies of Australia Antigen as a Virus

If Australia antigen is a virus related to hepatitis then it may be possible to transmit it to a susceptible animal host and perhaps produce the disease in the animal. We surveyed the sera of several hundred animals that included approximately 30 species. Australia antigen with apparently the same specificity as that found in humans was found only in the primates, and among the primates in only three species (chimpanzees, African green monkeys [vervets], and squirrel monkeys). Interestingly, it was not found in *Macaca mulatta*, the species most often used in nonhuman primate experimentation. We have initiated a series of experiments in which African green monkeys are inoculated with sera from patients with Australia antigen and with isolated Australia antigen. The animals are tested for the presence of Australia antigen in their blood, and for biochemical and pathological changes associated with liver damage. Since we have reason to believe that Australia antigen is associated with a chronic infection it will be necessary to observe these animals for a long time. Irving Millman is attempting to grow Australia antigen in tissue cultures of human and monkey origin. An immunofluorescent technique has been developed which we believe will be useful in identifying the antigen in cells and tissues.

Population Studies

Prior to these findings in respect of disease, we have amassed considerable information on the epidemiology of Australia antigen in nonhospitalized populations. Although the antigen is quite rare in normal Americans (Table I) it is not uncommon in several tropical popula-

TABLE III. DISTRIBUTION OF AUSTRALIA ANTIGEN IN SOME NONHOSPITALIZED ("NORMAL") POPULATIONS

Population	*Location*	*Number tested*	*Number positive*	*Per cent positive*
Americas				
Eskimos	Alaska (U.S.A.)	394	1	0.3
Indians, Athabascan	Alaska (U.S.A.)	204	0	0
Indians, Cashinahua	Peru	89	18	20.2
Indians, Haida	Canada	338	0	0
Indians, Maya	Yucatan	1,417	4	0.07
Indians, Mexico, various	Mexico	340	1	0.3
Indians, Navajo	U.S.	95	0	0
Indians, Quechua	Peru	102	0	0
Indians, Sioux	South Dakota (U.S.A.)	130	0	0
Negroes	Georgia, Maryland (U.S.A.)	607	0	0
Negroes	Brazil	119	3	2.5
Whites	Georgia, Maryland (U.S.A.)	896	0	0
Whites	Brazil	100	0	0
Africa				
"Bantu"	South Africa	72	2	2.8
"Colored"	South Africa	100	0	0
Pare	Tanzania	120	1	0.8
Tristan Da Cunha	Tristan da Cunba	42	0	0
Ghanaians	Ghana	95	9	9.5
Asia				
Indians	South India	127	3	2.4
Japanese	Japan (various)	1,034	5	0.5
Chinese	U.S.A., Taiwan	100	0	0
Taiwanese	Taiwan	23	3	13.0
Israelis	Israel	340	4	1.2
Jordanians	Jordan	40	0	0
Filipinos	Cebu, P.I.	764	37	4.8
Filipinos	Manila, P.I.	197	9	4.6
Vietnamese	Vietnam	128	8	6 3
Europe				
Finns	Finland	924	1	0.1
Lapps & Finnlapps	Finland	127	0	0
Italians	Italy	212	0	0
Greeks	Greece	857	15	1.8
Portuguese	Hawaii	44	1	2.2
Oceania				
Aborigines	Australia	1,807	38	2.1
Maoris	New Zealand	4	1	
Melanesians	New Guinea	166	6	3 6
Micronesians	Marshall Islands	474	34	7.2
Polynesians	Bora Bora	119	3	2.5
Polynesians	Hawaii	43	1	2.3

No corrections for age or sex have been made.

tions (Table III). For example, it occurs in about 5 per cent of Filipinos, 6 per cent of Vietnamese, 7 per cent of Micronesians, and in about the same frequency in several other groups of Malaysians, Micronesians, and Polynesians. It also occurs in Japanese and Mediterranean populations, but in lower frequency (i.e., about 1 per cent). West African

TABLE IV. DISTRIBUTION OF AUSTRALIA ANTIGEN BY AGE IN FOUR POPULATIONS*

	Marshall Islands			*Cebu*			*Manila*			*Cashinahua*		
Age	*No.*	*No. pos.*	*% pos.*	*No.*	*No. pos.*	*% pos.*	*No.*	*No. pos.*	*% pos.*	*No.*	*No. pos.*	*% pos.*
0-19	196	16	9.5	348	30	8.6	22	2	9.1	27	12	44.4
20-39	148	12	8.1	948	62	6.5	132	6	4.5	49	3	6.1
40-59	85	4	4.7	378	16	4.2	36	1	2.8	13	1	7.7
60+	67	1	1.5	128	3	2.3	6	0	0	0	0	—
Total	496	33	6.7	1,802	111	6.2	196	9	4.6	89	16	18.0

The Cebu population includes leprosy and nonleprosy individuals.
*Adapted from Blumberg *et al.*[28]

populations which have been tested also contain the antigen in high frequency. There is a definite pattern of age dependence in some of these populations. The frequency was invariably higher in the younger than in the older age groups (Table IV). We now have data, not shown in the table, that in very young people, i.e., under five years, the frequency is low. There was also a higher frequency in males than in females in nearly all the populations (Table V). Hepatitis, particularly chronic hepatitis, is endemic in many areas where Australia antigen is common.

The persistence of Australia antigen over a prolonged period of time has been studied in several populations. Australia antigen occurs in about 7 per cent of the residents of Rongelap Atoll, the Marshall Island district of the United States Trust Territory of the Pacific Islands.[28] Three hundred individuals have been tested since 1958. Of these, repeat samples were available on 270, 12 of whom were positive and the remainder negative. All the negatives were persistently negative. Of the positives, all were consistently positive with two exceptions (Figure 4). We also have data on a group of Down's syndrome patients extending over three years, and in these as well the trait is, in general, persistent. We have data from the island of Cebu extending over the course of three years; the presence of the antigen is often persistent. An individual with Australia antigen at one point in time has a very much higher probability of having the antigen at second testing than an individual who did not have the antigen at the first observation. From this we can conclude that the antigen appears to be persistent in the patients with Down's syndrome and in certain tropical populations.

TABLE V. DISTRIBUTION OF AUSTRALIA ANTIGEN BY SEX IN FOUR POPULATIONS*

	Total number	*Number positive*	*Per cent positive*
Marshall Islands			
Male	243	19	7.8
Female	226	14	6.2
Total	496	33	6.7
Cebu			
Male	430	27	6.3
Female	334	10	3.0
Total	764	37	4.8
Manila			
Male	138	6	4.3
Female	59	3	5.1
Total	197	9	4.6
Cashinahua			
Male	45	10	22.2
Female	44	6	13.6
Total	89	16	18.0

The Cebu populations include only individuals without leprosy since there is an increased frequency of lepromatous leprosy in males and an increased frequency of Australia antigen in lepromatous leprosy male patients (Blumberg *et al.*[28]). The male-female difference is statistically signicant for the Cebu populations ($X^2 = 4.4$, $0.05 > P > 0.025$) but not for the others.

*Adapted from Blumberg *et al.*[28]

INTERACTION OF HOST AND ENVIRONMENT

There is evidence that both host and environmental factors have an important bearing on the chronicity of Australia antigen in some diseases and population groups. Patients with Down's syndrome who resided in large institutions (1,100 or more residents), small institutions (20 to 50 patients), and patients living at home were tested for Australia antigen.[25] It was found in 28 per cent of patients in large institutions, 3 per cent of patients in small institutions, and not present in outpatients (Table VI). This is compatible with the notion that an environmental factor, such as an infectious agent, is responsible for the presence of the antigen, and that it is more readily spread in the crowded conditions which exist in large institutions.

Within the large institutions patients with Down's syndrome, other mentally retarded patients without Down's syndrome, and employees of the institution were tested. The antigen was found in 28 per cent of the patients with Down's syndrome, in 3 per cent of the other patients,

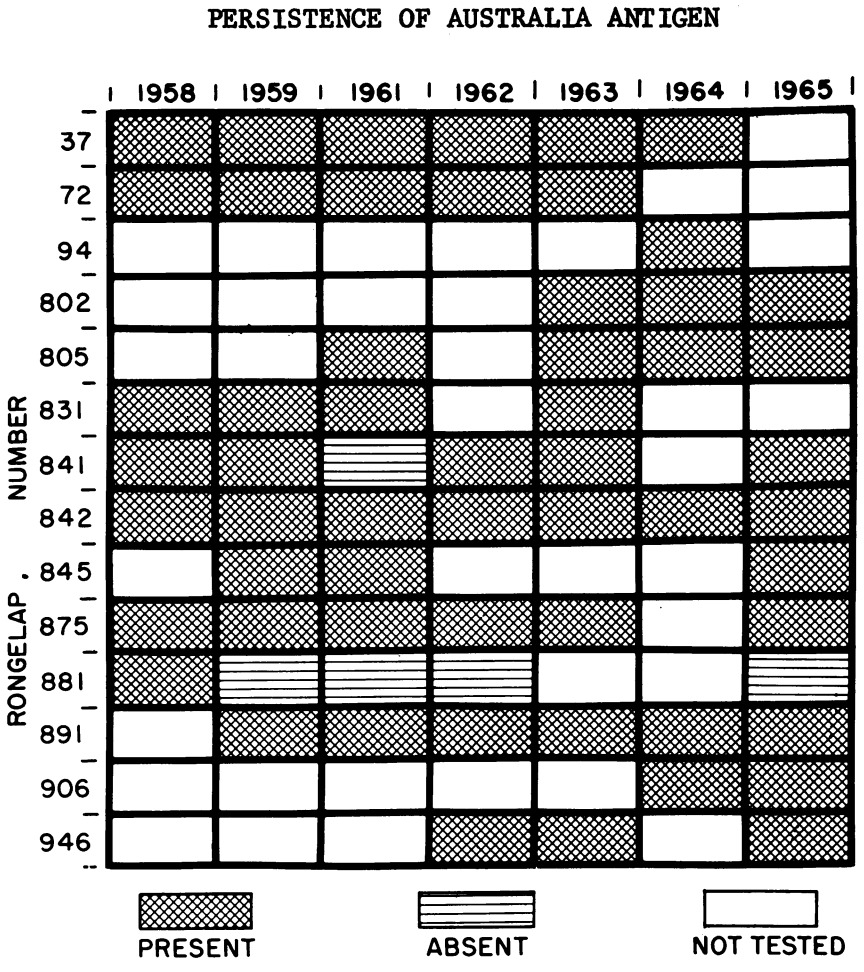

Fig. 4. Studies on the persistence of Australia antigen in blood samples collected serially from apparently normal individuals from Rongelap. The subject numbers are shown on the left. The reactions of sera collected in the years indicated at the top are given in the body of the figure. Adapted from Blumberg *et al.*[28]

and not in any of the employees. The patients are not segregated according to diagnosis, and the employees for the most part have intimate daily contact with the patients. From this it appears that there are host factors nearly unique to the patients with Down's syndrome in the institutions, which results in a high frequency of chronic "infection" with Australia antigen. This does not rule out the possibility that the other patients and the employees could contract acute hepatitis with the transient emergence of Australia antigen. A sample taken at a single

TABLE VI. DISTRIBUTION OF AUSTRALIA ANTIGEN IN PATIENTS WITH DOWN'S SYNDROME, IN OTHER MENTALLY RETARDED PATIENTS, AND IN NORMAL PERSONS IN LARGE INSTITUTIONS, IN SMALL INSTITUTIONS, AND IN PERSONS NOT RESIDING IN INSTITUTIONS

	Australia antigen frequency					
	Host					
Environment	*Down's syndrome*		*Other mental ret.*		*Normal*	
Large institutions	86/310	28%	6/188	3%	0/114	0%
Small institutions	1/33	3%	—		—	
Not in institutions	0/45	0%	0/10	0%	2/2,412	

point in time would not detect any (or many) of these unless it happened to be taken early in the course of the disease or during an epidemic.

FAMILY STUDIES

In respect of the host factors revealed by the Down's syndrome project, early in the investigation the family aggregation of Australia antigen had been studied. We had tested about 1,000 sera collected on Cebu in 1964. Of these, about 60 were positive for Australia antigen. We were able to obtain 78 bloods from the family members of 13 of these positive individuals and 153 bloods from the family members of 40 individuals who did not have Australia antigen (Table VII).[28] Among the family members of the "positive" propositi, 24 were positive or, if the index cases are deducted, 11 of 65, that is, 17 per cent. Among the family members of the individuals without Australia antigen, 2 of 153 or 2 of 113 (2 per cent) with the index cases subtracted, were positive; there is strong family aggregation of the trait in this population. Family aggregation is consistent with an infectious hypothesis, but it is also consistent with a genetic hypothesis. The segregation of the trait in the families showed an interesting pattern. There were many instances in which sibs were positive but often neither of the parents were positive. Using C. A. B. Smith's method of segregation analysis, we tested the hypothesis of autosomal recessive inheritance, i.e., that the presence of Australia antigen (phenotype Au[1]) was determined by a gene designated Au^1. Homozygotes for this postulated gene (Au^1/Au^1) are positive and heterozygotes or homozygotes for the alternative gene ($Au^1/$

TABLE VII. AUSTRALIA ANTIGEN IN THE FAMILIES OF PERSONS FROM CEBU IDENTIFIED THROUGH AUSTRALIA ANTIGEN-POSITIVE AND AUSTRALIA ANTIGEN-NEGATIVE INDEX CASES*

Families studied	*With positive index case*	*With negative index case*
Number of families	13	40
Index cases included:		
Family members	78	153
Positive Australia antigen	24	2
Index cases subtracted:		
Family members	65	113
Positive Australia antigen	11	2
Per cent positive	16.92	1.76

The probability for this distribution is $P = 3.32 \times 10^{-4}$ by Fisher's exact method.
*Adapted from Blumberg *et al.*[28]

Au; *Au/Au*) are negative. The segregation data on families from Cebu and elsewhere were highly consistent with this hypothesis (Table VIII).

Despite this close fit with the segregation pattern predicted by the autosomal recessive hypothesis, there could be another explanation for these findings. For example, if the presence of Australia antigen were due entirely to an infectious process and if the attack rate were higher in children than in adults, we should expect a distribution similar to that found in the families. Proof of the genetic hypothesis must await additional family studies, which are now in progress. If the genetic hypothesis is sustained, then this implies that there is a host susceptibility to persistent "infection" with Australia antigen and that this susceptibility is inherited. If this is true, then in areas where the gene and infectious agent are common, the antigen would appear to follow Mendelian segregation.

This study illustrates the difficulty which may arise in distinguishing between an infectious and genetic etiology of a trait. This is especially true of autosomal recessive traits of relatively low frequency where critical matings (ie., between two Au positive parents) are very rare. In the case of Australia antigen and perhaps other traits both hypotheses may be correct.

LEUKEMIA

We have now studied about 600 sera from 385 different patients with various forms of leukemia.[11-12] The antigen occurs in 14 per cent

TABLE VIII. SEGREGATION OF Au(1) IN FAMILIES WITH AT LEAST ONE Au(1) (POSITIVE) CHILD*

Number of children in family	*Number of families*	*Observed number of recessive children*	*Expected number of recessive children*	*Variance*
Cebu families: mating type, positive × negative				
c	m_c		$m\,a_{c\,c}$	$m\,b_{c\,c}$
2	1	1	1.333	0.222
3	2	3	3.428	0.980
4	1	3	2.133	0.782
Total	4	$R_1 = 7$	$E_1 = 6.894$	$V_1 = 1.984$
Cebu families: mating type, negative × negative				
c	M_c		$M\,A_{c\,c}$	$M\,B_{c\,c}$
3	2	2	2.594	0.526
4	2	3	2.926	0.840
5	1	1	1.639	0.592
6	2	3	3.650	1.552
8	1	4	2.223	1.172
Total	8	$R_2 = 13$	$E_2 = 13.032$	$V_2 = 4.682$
All families: mating type, positive × negative				
c	m_c		$m\,a_{c\,c}$	$m\,b_{c\,c}$
1	1	1	1.000	0.000
2	1	1	1.333	0.222
3	3	6	5.142	1.470
4	1	3	2.133	0.782
5	1	1	2.581	1.082
Total	7	$R_1 = 12$	$E_1 = 12.189$	$V_1 = 3.556$
All families: mating type, negative × negative				
c	M_c		$M\,A_{c\,c}$	$M\,B_{c\,c}$
1	8	8	8.000	0.000
2	3	4	3.429	0.366
3	3	3	3.891	0.789
4	3	5	4.389	1.260
5	2	3	3.278	1.184
6	3	5	5.475	2.328
7	1	1	2.020	0.970
8	1	4	2.223	1.172
Total	24	$R_2 = 33$	$E_2 = 32.705$	$V_2 = 8.069$

Computations by the method of Smith for Cebu families and for all families. In each case, the observed number (R_1, R_2) is very close to the expected (E_1, E_2), and the difference between them is always less than the variance (V_1, V_2).

*Adapted from Blumberg *et al.*[28]

of patients with acute myelogenous leukemia, in 12 per cent of those with acute lymphocytic leukemia, and in 10 per cent of those with chronic lymphocytic leukemia, but it does not occur in the 63 patients with chronic myelogenous leukemia who have been tested. In one instance the antigen was found in a young patient with "preleukemia" who subsequently developed acute myelogenous leukemia. Although our evidence is not yet complete, the antigen appears in patients who have received all modes of treatment (chemotherapy, x-ray therapy, transfusion therapy) and in patients who have never received transfusions or radiation therapy. Nearly all of these leukemia patients were hospital inpatients, and we do not have epidemiological data on the presence of the antigen early in the disease. We now want to know if the antigen is associated with some feature connected with hospitalization and treatment or is present in patients prior to the beginning of treatment.

Other Specificities

The experiments described thus far have been done with antisera with apparently the same specificity; these have been referred to as anti-Au(1) antisera. Antisera which appear to contain other specificities have been developed by the immunization of rabbits with human sera which cross-react with Australia antigen but in addition have specificities different from those in Au(1). These are described in detail in a forthcoming publication.[29] The second major specificity has a population distribution quite different from that of Au(1). In particular it occurs in a much higher frequency of patients with known or suspected hepatitis (71 per cent) than does the first specificity (20 per cent), and it occurs in nearly 100 per cent of patients who receive very large numbers of transfusions. It also occurs in a very high percentage of patients with Down's syndrome (75 per cent of 155 patients). The relation of these specificities to hepatitis is not yet clear. It is conceivable that they react with other specificities of the antigen and may be more useful in detecting occult carriers.

Immune Mechanism: Leprosy

An intriguing feature of the observations is the specific group of diseases in which the antigen is found. In addition to the leukemias and Down's syndrome, which have already been discussed, the antigen has been found in high frequency in patients with lepromatous leprosy.[15]

A total of 1,796 individuals were tested on the island of Cebu. Nine point 4 per cent (9.4 per cent) of 584 patients suffering from lepromatous leprosy had Australia antigen; 9.9 per cent of 71 borderline cases, which are similar to lepromatous cases; 3.4 per cent of 377 tuberculoid cases; and 4.8 per cent of 764 persons without leprosy. The difference in the prevalence of the cases in the lepromatous and borderline group combined was significantly higher than in the tuberculoid and non-leprosy groups, and this difference persisted when the samples were corrected for age and sex. Patients with lepromatous leprosy have generalized impairment of delayed tissue hypersensitivity.[30]

Hypotheses

A feature common to those apparently disparate diseases is that they present deficiencies in the immune mechanism. This could result in susceptibility to chronic infection with Australia antigen (presuming it to be infectious) whereas if infection occurred in other immunologically normal individuals the infection would be transient.

A major objective of our work now is to determine if Australia antigen is in fact a virus. Although its appearance under the electron microscope is consistent it will be necessary to show that it has other qualities of a microorganism. The studies outlined above on tissue culture, animal transmission, immunofluorescence, and others should help to answer this question.

There are now two major hypotheses which require testing:

1) Patients with leukemia, Down's syndrome, and lepromatous leprosy have a deficiency in their immune mechanism. In some populations the deficiency may be inherited. This results in chronic infection with a "carrier virus" associated with Australia antigen. This infection is associated with chronic or acute hepatitis.

2) Australia antigen is an infectious agent which in some people causes hepatitis and, in others, some kinds of leukemia. This is analogous to the findings of G. Henle *et al.*[31] who suggested that a single infectious agent may be responsible for infectious mononucleosis in some people and for Burkitt's lymphoma in others.

We hope that experiments now in progress will help to answer these questions.

ACKNOWLEDGMENTS

This work has been done in collaboration with Drs. M. Bayer, Betty Jane S. Gerstley, C. Levene, and Liisa Melartin, and with M. Cronlund and Miss Barbara Werner.

Addendum. Recently Prince, who had assisted with the isolation of Australia antigen,[21] reported an antigen ("SH") that occurred in the blood of patients with serum hepatitis during the incubation period of the disease.[31] He later identified this antigen as Australia antigen.[33] His data constitute further evidence for the association of Australia antigen with hepatitis.

REFERENCES

1. Allison, A. C. and Blumberg, B. S. An isoprecipitation reaction distinguishing human serum protein types. *Lancet 1*:634, 1961.
2. Blumberg, B. S. and Allison, A. C. Studies on the isoprecipitin-determined human serum polymorphism. *Proc. Second Intern. Congr. Human Genet.* (Rome), 1961, pp. 733-736.
3. Blumberg, B. S., Dray, S. and Robinson, J. C. Antigen polymorphism of a low-density beta-lipoprotein. Allotypy in human serum. *Nature 194*:656, 1962.
4. Blumberg, B. S., Bernanke, D. and Allison, A. C. A human lipoprotein polymorphism. *J. Clin. Invest. 41*:1936, 1962.
5. Blumberg, B. S., Alter, H. J., Riddell, Nancy and Erlandson, Marion. Multiple antigenic specificities of serum lipoproteins detected with sera of transfused patients. *Vox Sang. 9*:128, 1965.
6. Vierucci, A., Morganti, G. and Varone, D. New anti-beta-lipoprotein sera in transfused children with thalassemia. *Vox Sang. 11*:427, 1966.
7. Butler, R. Polymorphisms of the human low-density lipoproteins. *Vox Sang. 12*:2, 1967.
8. Hirschfeld, J. The Ag-system. Comparison of different isoprecipitin sera. *Ser. Haemat. 1*:38, 1968.
9. Blumberg, B. S. Polymorphisms of serum proteins and the development of isoprecipitins in transfused patients. *Bull. N. Y. Acad. Med. 40*:377, 1964.
10. Blumberg, B. S., Alter, H. J. and Visnich, S. A "new" antigen in leukemia sera. *J. A. M. A. 191*:541, 1965.
11. Blumberg, B. S., Gerstley, B. J. S., Hungerford, D. A., London, W. T. and Sutnick, A. I. A serum antigen (Australia antigen) in Down's syndrome, leukemia and hepatitis. *Ann. Intern. Med. 66*:924, 1967.
12. Sutnick, A. I., London, W. T. and Blumberg, B. S. Leukemia and Australia antigen. (In preparation.)
13. Melartin, L. and Panelius, M. Occurrence of Australia antigen in Finnish mongolism patients. *Ann. Med. Exp. Fenn. 45*:157, 1967.
14. Blumberg, B. S. and Alter, H. J. Precipitating antibodies against a serum protein ("Australia antigen") in the serum of transfused hemophilia patients. *J. Clin. Invest. 44*:1029, 1965.
15. Blumberg, B. S., Melartin, L., Lechat, M. and Guinto, R. S. Association between lepromatous leprosy and Australia antigen. *Lancet 2*:173, 1967.
16. Blumberg, B. S. and Riddell, N. M. Inherited antigenic differences in human serum beta-lipoproteins. A second antiserum. *J. Clin. Invest. 42*:867, 1963.
17. Melartin, L. and Blumberg, B. S. Production of antibody against "Australia antigen" in rabbits. *Nature 210*:1340, 1966.
18. London, W. T., Blumberg, B. S. and Sutnick, A. I. Australia antigen and acute hepatitis. *Ann. Intern. Med.* In press, 1968.

19. Hoagland, R. J. and McCluskey, R. T. Hepatitis in mononucleosis. *Ann. Intern. Med. 43*:1019, 1956.
20. Alter, H. J. and Blumberg, B. S. Studies on a "new" human isoprecipitin system (Australia antigen). *Blood 27*:297, 1966.
21. Bayer, M. E., Blumberg, B. S. and Werner, B. Particles associated with Australia antigen in the sera of patients with leukemia, Down's syndrome and hepatitis. *Nature 218*:1057, 1968.
22. Sherlock, S. *Diseases of the Liver and Biliary System.* Philadelphia, Davis, 1966.
23. W.H.O. Expert Committee on Hepatitis. Geneva, WHO, 1964, p. 25.
24. Sutnick, A. I., London, W. T. and Blumberg, B. S. Australia antigen, Down's syndrome and hepatitis. *J. Clin. Invest. 46*:1122, 1967 (Abstr.).
25. Sutnick, A. I., London, W. T., Gerstley, B. J. S., Cronlund, M. M. and Blumberg, B. S. Anicteric hepatitis associated with Australia antigen. *J.A.M.A.* In press, 1968.
26. Okochi, K. Ag(x,y) in Japanese and preliminary report on an isoprecipitin. *J. Jap. Soc. Blood Trans. 14*:151, 1967.
27. Okochi, K. and Murakami, S. Observations on Australia antigen in Japanese. *Vox Sang.* In press, 1968.
28. Blumberg, B. S., Melartin, L. Guinto, R. A. and Werner, B. Family studies of a human serum isoantigen system (Australia antigen). *Amer. J. Hum. Genet. 18*:594, 1966.
29. Levene, C. and Blumberg, B. S. Multiple specificities of a serum antigen ("Australia antigen"). In preparation, 1968.
30. Waldorf, D. S., Sheagren, J. N., Trautman, J. R. and Block, J. B. Impaired delayed hypersensitivity in patients with lepromatous leprosy. *Lancet 2*:773, 1966.
31. Henle, G., Henle, W. and Diehl, V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. *Proc. Nat. Acad. Sci. U.S.A. 59*:94, 1968.
32. Prince, A. M. An antigen detected in the blood during the incubation period of serum hepatitis. *Proc. Nat. Acad. Sci. 60*:814, 1968.
33. Prince, A. M. Relation of Australia and SH antigens. *Lancet 2*:462, 1968.